

HERPETOMONAS STRATIOMYIAE, N.SP., A FLAGELLATE PARASITE OF THE FLIES, *STRATIOMYIA CHAMELEON* AND *S. POTAMIDA*, WITH REMARKS ON THE BIOLOGY OF THE HOSTS

BY

H. B. FANTHAM, D.Sc. (LOND.), B.A. (CANTAB.)

(LECTURER ON PARASITOLOGY, LIVERPOOL SCHOOL OF TROPICAL MEDICINE)

AND

ANNIE PORTER, D.Sc. (LOND.), F.L.S.

(QUICK LABORATORY, CAMBRIDGE)

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I. INTRODUCTION

The interesting flies, *Stratiomyia** *chameleon* and *S. potamida*, are fairly common in certain districts where the drainage of the land is poor and where boggy areas with somewhat rank vegetation occur. Both the larva, pupa and imago of the insects are sometimes parasitised by a small Protozoön, belonging to the genus

* The original generic name of the host flies was *Stratiomys*. Many modern authorities prefer the form *Stratiomyia*, which has been adopted in this paper. The *Stratiomyia* were obtained near Cambridge, and one local name for them is Chameleon flies.

Herpetomonas, and presenting certain differences from other common *Herpetomonads* that warrant its inclusion as a new species. The chief interest of the parasite lies in its relation to the life-history of the host, and thus a brief note on the biology of the hosts is considered advisable.

We do not propose to enter into great cytological detail regarding the structure of the flagellate, nor to engage in fruitless discussion as to the nomenclature of such parasites, but rather to consider the flagellate in relation to the life-history of the host. The relation of insect flagellates to their hosts' life-cycle is a most interesting though difficult subject, on which little or nothing has been done in most cases up to the present.

II. THE BIOLOGY OF THE HOST FLIES, *STRATIOMYIA CHAMELEON* AND *S. POTAMIDA*

(a) *Habits*. The larvae of these insects are very characteristic, worm-like, legless organisms, armed with powerful jaws by which they drag the body forwards, progression being aided by bristles attached to each segment. The larger larvae examined varied from $\frac{3}{4}$ inch to $2\frac{1}{2}$ inches in length at will, as the abdomen can be retracted telescopically. Their colour varies with that of the mud and decaying vegetation among which they live, yellowish, brown and green being common hues. Twenty-five to thirty respiratory tail filaments are present and are applied by the larva to the surface film, so that in deep water the larva is suspended vertically, head downwards.

The pupae are found near to the surface in the mud fringing the pond or stream. The larval skin is retained and the much smaller pupa is enclosed within it. The period of pupation is short.

The imago is somewhat bee-like and is blackish in colour with bright yellow markings. Eggs are laid on aquatic plants just above the level of the water, and the larvae either escape into the water, or are hatched beneath the surface into which the plants finally fall.

(b) *Food*. The food of the imago seems to be sweet vegetable material. The larvae, so far as can be ascertained by direct observations extending over three years, are vegetable feeders. The contents of the alimentary tracts of those examined have never

contained blood or other recognisable animal matter such as muscle or chitinous tissue that could be obtained from insects or Crustacea. Small organisms such as Protozoa may be ingested, but no proof that such was the case occurred. A typical set of examinations of 50 larvae from 10 different localities, representing about one-third of the total number examined, gave the following results:—

From the first locality, seven larvae varying from $\frac{3}{4}$ inch to $2\frac{1}{4}$ in. long, were obtained. Two were infected with *Herpetomonas*, one infected larva was $1\frac{1}{2}$ in. long, the other $\frac{3}{4}$ in. The large vegetation of the locality comprised grass, *Veronica beccabunga*, *Myosotis palustris*, various algae, and many rotting leaves. The contents of the food canals of the larvae were portions of *Chara*, *Cladophora*, *Spirogyra* and *Zygnema*.

Ten larvae from the second locality were from 1 in. to 2 in. long. None were infected. Grass and *Veronica beccabunga* constituted the larger vegetation. There were fewer large algae than in the first locality. The alimentary canals of these larvae contained algae, chiefly species of *Closterium*, *Penium*, *Micrasterias* and *Cosmarium*.

Six larvae from a third place varied from $1\frac{1}{4}$ in. to $2\frac{1}{2}$ in. long. Like the previous set, they were uninfected. Their gut contents consisted almost entirely of *Gonium* sp., and the larger vegetation on the spot comprised grass, *Myosotis* and Common Hemlock.

A small pond, whose bordering vegetation consisted of grass and *Myosotis*, and in which some fine *Hottonia* grew, yielded seven larvae. These contained many algae, chiefly species of *Scenedesmus* and *Selenastrum*. No parasites were found in the larvae.

Six larvae were obtained from the fifth locality. All were vigorous, and varied from 1 in. to $2\frac{1}{4}$ in. in length. Grass, *Myosotis* and algae were abundant. The food passages of the larvae contained algae, chiefly species of *Navicula*, *Diatoma*, *Synedra*, *Gyrosigma* and *Nitzschia*. No herpetomonads were found.

Some of the smallest larvae were obtained from the sixth locality. They varied from $\frac{1}{2}$ in. to 2 in. in length. Seven were examined. A larva, 1 in. long, had a slight infection with *Herpetomonas stratiomyiae*, the rest were normal. Rotting leaves occurred in the water and hemlock, *Veronica beccabunga* and grass constituted the larger vegetation. Algae, chiefly species of *Navicula*, *Denticula* and *Nitzschia*, together with fragments of leaves, were found in the alimentary canals of the larvae.

Three larvae only were obtained from the seventh locality, whose vegetation was the same as that of the sixth. The gut contents of the larvae also were similar. None were infected.

One specimen, $1\frac{3}{4}$ in. long, obtained from a small brook partly choked with *Potamogeton crispus* and large algae, contained no parasites. Its gut contents included species of *Scenedesmus* and *Cosmarium*, together with fragments of *Chara*.

One larva, 2 in. long, was obtained from a ditch fringed with willowherb. Its gut contained numerous specimens of *Cosmarium* and a very few *Herpetomonads* also were present.

Two larvae from the tenth locality were uninfected. The chief plants growing in the mud were willowherb and water dropwort, while the food contents of the larvae consisted of species of *Cosmarium* and *Navicula*.

The presence of infected larvae coincided to some extent with the quantity of decaying matter that was present; but sometimes larvae obtained from the foulest sources (not those detailed) were as free from infection as those from relatively uncontaminated places. Larvae were in greatest abundance where much decaying material was present.

Altogether, nearly 150 larvae were examined, and the percentage infected with *Herpetomonas* was only about 3. The percentage of infection in the pupae examined was somewhat higher.

III. MATERIAL AND METHODS

The larvae, pupae and imagines of *Stratiomyia*, comprising both bred insects and those caught at large, have been used during this investigation. The *Herpetomonas* has been found most abundantly in the digestive tract of the larva. The alimentary canal has been dissected wherever possible, placed in physiological salt solution and examined in serial teased portions. The pupal condition usually prevented dissection of the alimentary canal, but smears of the central part of the body were sufficient to allow of identification of the parasite. Much time has been spent in observing the living *Herpetomonas*. Osmic acid followed by absolute alcohol, Bouin's fluid and Flemming's solution have been used for fixatives, and Giemsa's solution, iron haematoxylin and glycerin haematin were used as stains. The paraboloid condenser and stereoscopic oculars have also been used and found of much service in determining depths of granules, chromatin particles and similar structures.

V. THE LIFE HISTORY OF *HERPETOMONAS STRATIOMYIAE*

The life-cycle of the herpetomonad may be divided into two main phases: (1) a resting phase, (2) an active, multiplicative phase. The resting phase again can be sub-divided according to the period at which it is present in the host. Infection is by the contaminative method, and freshly ingested resting forms of the parasite about to develop into the active form are best described as the preflagellate stages, while the forms produced by the preparation of the full flagellate for extra-corporeal life are more accurately described as post-flagellate forms. Naturally the post-flagellate form produced in the first host becomes the pre-flagellate organism in the second host.

A. THE HERPETOMONAD IN THE LARVA OF *STRATIOMYIA*

The preflagellate stage of *Herpetomonas stratiomyiae* differs from that of most herpetomonads in being elongate oval in shape (Pl. XLI, figs. 1, 2). Its length varies from 5.5μ to 8μ , while

its breadth is about 3μ . The cytoplasm is clear in life or slightly granular. The nucleus shows as an oval, refractile vacuole, the blepharoplast as a bar-like rod. They both lie relatively near the surface. Sometimes the end of the body near the blepharoplast appears somewhat more refractile in life than the distal end. Such an area, when stained, proved to be the chromatophile vacuole-like area from which the flagellum originates. The nucleus presents a definite nuclear membrane, seen best in haematin-stained preparations. The chromatin varies in its arrangement, sometimes appearing as a more or less central concentrated karyosome (fig. 2), at other times being scattered as granules in the nucleoplasm (fig. 1). The bar-like blepharoplast stains deeply. The formation of a flagellum occurs very rapidly, and hence it is difficult to find stages in which the chromatophile area is present unless the host is dissected just at the time preceding the flagellation of the parasites. Division occurs among the preflagellates (figs. 4, 5), and will be described later.

The flagellates (figs. 10-17) vary in size, the length including the flagellum being from 26.6μ to 57μ , while the breadth is from 2μ to 3.6μ . The flagellum itself may occasionally reach 38μ long. The non-flagellate or posterior end of the flagellate is elongate but somewhat blunted; the flagellar or anterior end is somewhat rounded, with the flagellum projecting from it. As with the preflagellate forms, the nucleus is oval and shows cyclical development. Relatively young flagellates, or flagellates that have formed rapidly from the preflagellate forms, often possess a vesicular nucleus with a central (figs. 15, 16) or slightly excentric (fig. 12) karyosome. Older parasites, those of slow growth and forms about to divide, have their nuclear grains of chromatin evenly distributed (figs. 11, 14). The blepharoplast is curved, rod-like or oval, and, except in dividing forms, is homogeneous in structure. Chromatoid granules may be present in some flagellates, usually forming small grains in the post-nuclear part of the body (figs. 11, 13).

Multiplication. The increase in numbers of the parasites within the host is brought about by longitudinal division, which can take place in either the preflagellate (figs. 3-5) or flagellate (figs. 18-22) stages. The division of the preflagellates is initiated by that of the blepharoplast, which becomes dumb-bell shaped (fig. 3),

showing two concentrations of chromatin, one at each end. The heads of the dumb-bell remain attached to one another for some time by a strand of chromatin. The constriction of the nucleus rapidly follows that of the blepharoplast. The future flagellar end then becomes cleft (fig. 4), and the cleft extends backwards. As soon as parts of the two daughter organisms are free, rapid movements occur, each free part diverging from the other and twisting on itself as it does so, until the two organisms come to lie in almost a straight line (fig. 5). Violent movements precede the final separation, and this is usually succeeded by relative quietness on the part of both the daughter forms, which usually do not move away until some seconds and, occasionally, minutes have elapsed. When movement occurs it is not active as with the flagellates, but consists of slow undulations of the body, which gradually propel the daughter forms forwards.

The stages of division of the flagellate (figs. 18-22) are like those of the preflagellate form, but the division of the blepharoplast and flagellum takes place almost simultaneously (figs. 19, 20), while nuclear division is often delayed, and at times, cleavage of the cytoplasm has commenced (fig. 20) before the nucleus has completely divided. The movements of the two parts of the dividing organism (figs. 21, 22) are very active, and the final separation is effected more quickly than that of the dividing preflagellate, the lashing of the daughter flagella being of material aid. The two newly-formed individuals swim away rapidly after separation. Examination of stained specimens has been confirmatory in all respects of the above observations made on the living organisms.

The formation of a post-flagellate form (figs. 23-36) is brought about by the gradual retraction and absorption of the flagellum (figs. 23-27) and the concentration of the body until it forms an oval or rounded form, which secretes a thin, closely adherent cyst around itself. When the assumption of the post-flagellate form is complete, there is no trace of the flagellum remaining as such. Even its root (or rhizoplast) disappears, the chromatin of it appearing to be dissolved and concentrating, in some cases, in a small part to form a chromatoid area, as well as diffusing generally through the body substance, as shown by the staining reactions of the cyst. The post-flagellate is *Leishmania*-like (figs. 32-36), of a somewhat

elongate type. The nucleus may, or may not, show a karyosome, for as with the flagellate, the structure of the nucleus varies. If there is a karyosome in being at the time of encystment, the nucleus of the post-flagellate shows the same feature (figs. 34, 36). Should encystment follow rapidly on division, the nucleus is almost homogeneous (figs. 33-35). Much discussion has arisen recently as to the existence of specially differentiated portions of the blepharoplast, described variously as karyosomes and centrioles. In some cases, by the use of iron-haematoxylin, some granules have been demonstrated, but it is admitted that the existence of these granules depends for its demonstration on the degree of removal of the stain by the iron alum. They are not found when other stains are employed. The basing of argument on the fleeting appearances produced by too great or too little differentiation—the degree of differentiation that is ‘accurate’ being an absolutely individual opinion—is merely vexatious, and does not tend to the advancement of knowledge on really scientific lines.

B. THE HERPETOMONAD DURING PUPATION

As the period for pupation of the host approaches, the post-flagellate forms of the parasite leave the gut of the larval host and pass out with the faeces. Rapid flagellation of any preflagellates that are present occurs, together with division of the flagellates. The organisms seem to collect in the middle-third of the gut and place themselves with their flagella towards the centre of its lumen, their aflagellar ends being in contact with its wall. The latter is very frail at this time, and the flagellates pass through it with ease. They thus reach the haemocoel, and there they swim for a short time in their normal position with their flagella forwardly directed. Division occurs but infrequently. The flagellates (figs. 23-25) gradually slow their movements and then rapidly concentrate their substance (fig. 27). They soon become typical, post-flagellate forms (figs. 27-31), with distinct nuclei and blepharoplasts, and very thin cyst walls. These organisms are, perhaps, somewhat more frail-looking than those found in the rectum and faeces of the larva (figs. 32-36). At first they are intermingled with the numerous wander cells found in the pupae at this time, but they seem to prefer the more fluid parts of the host. The result is that, in a late pupa

in which the musculature has become fairly well developed, the post-flagellates lie in the more fluid medium, internal to the region of differentiated muscles. At times, they have been found crowded together roughly at the centre of the pupa, and seem to be united temporarily by gelatinous material. When the differentiation of the alimentary canal is in progress, the parasites in most cases become surrounded by it and can be found attached by the gelatinous secretion to the cells of the gut. They remain thus until the emergence of the imago. Four out of fifteen pupae were found infected, but in one only was the infection heavy.

C. THE HERPETOMONAD IN THE IMAGO

The number of infected imagines is much smaller than that of infected larvae, though neither are abundant. The occurrence of larvae in numbers in one district enables them to become infected with post-flagellates from their neighbours with a certain amount of ease, as they swallow the cysts with their food. But all infected larvae do not give rise to infected pupae, while the latter may have but a slight infection that fades out and disappears in the adult. The habits of the imagines do not readily allow of them acquiring infection *ab initio*, and consequently the numbers found infected have been extremely small. The parasites have been found in all stages. Non-flagellates, like those in the pupa, have been found in the gut near the junction of the thorax and abdomen. They rapidly form flagellates that spread quickly throughout the whole length of the gut, while post-flagellates occur in the rectum and faeces. No stages of the parasite have been observed up to the present in the genital organs and but few in the haemocoel of the host. When there is haemocoelic infection, there are two possible means of origin: (1) the parasites may have remained there from the pupation period, or (2) they may have penetrated from the gut as flagellates. As they are capable of moving and developing fully in the haemocoelic fluid, hereditary infection is possible, though we are not in a position to pronounce on this with certainty, owing to the small number of infected flies that have been examined and to the difficulty of obtaining eggs. We hope to continue this part of the work in the future.

V. MODE OF INFECTION

The mode of infection is contaminative originally. The larvae acquire post-flagellate forms of the *Herpetomonas* with their food. The cysts may have been derived either from already infected larvae or from the dejecta of adult flies. The faeces of a larva on one occasion contained active flagellates as well as post-flagellate forms. As the flagellates lived for some hours in ordinary water, it is possible that they could do so in nature, and act as an additional source of infection of new hosts.

The parasite can persist through the metamorphosis of the host and the imagines thus emerge infected. It is possible, though we think it is probably exceptional, that an adult insect may become infected by sucking plant juices contaminated with excrement from other imagines, and thus acquiring the post-flagellate stages of the parasite.

VI. CONCLUDING REMARKS

The flagellate parasite of *Stratiomyia chameleon* and *S. potamida* is a member of the genus *Herpetomonas* as originally defined. It differs from *H. pediculi* (Fantham, 1912) from the body louse. *Pediculus vestimenti*, in its characteristic, oval, preflagellate form. *Herpetomonas jaculum* from *Nepa cinerea* is similar to *H. stratiomyiae* in appearance, but again the preflagellates of *H. stratiomyiae* are distinctive, the same feature differentiating the parasite from *H. lygaei* and *H. culicis*. It also shows differences from *H. muscae domesticae*, *H. ctenocephali*, *H. ctenophthalmi* and other known *Herpetomonads*. In consequence of these morphological differences, and as it is the first time that such a parasite has been recorded from the *Stratiomyidae*, the organism has been named *Herpetomonas stratiomyiae*. We believe that this account is the first detailed record of the behaviour of an insect flagellate during the pupation of its host, a feature which gives a special interest to *Herpetomonas stratiomyiae*.

The study of the life-cycle of such herpetomonad flagellates of insects is of the utmost importance in view of the recent experiments of Laveran and Franchini (1913) on the successful experimental inoculation of *H. ctenocephali* (Fantham, 1912), from the gut of the dog-flea, into mice and other mammals. Such researches suggest an experimental leishmaniasis in the making.

VII. SUMMARY

1. *Herpetomonas stratiomyiae*, n. sp., is a parasite of the larvae, pupae and imagines of the flies, *Stratiomyia chameleon* and *S. potamida*.

2. The herpetomonads present three stages in their life-history, (a) a preflagellate stage, (b) a flagellate stage and (c) a post-flagellate stage.

3. Preflagellate forms are characteristically oval, with elongate nucleus and distinct blepharoplast. The flagellates vary from 26.6μ to 57μ in total length. The post-flagellates, as found in the hind gut of the larvae, are oval, with somewhat thicker walls than the preflagellates.

4. Multiplication is by longitudinal division and can occur in any phase of the life-history of the organism.

5. Just prior to pupation, the flagellates migrate from the gut of the larva into the haemocoel where they gradually become motionless, withdraw their flagella and assume the post-flagellate form. They collect in the more fluid parts of the body of the pupa, and, ultimately, as a result of this, they become enclosed in the gut of the adult.

6. The imago, if formed from an infected pupa, usually emerges from the pupa case infected with non-flagellate forms of the herpetomonad. The development of the non-flagellate into the flagellate form is rapid, and soon all stages of the organism are present.

7. Infection of the larvae is contaminative, that of the pupa is transmitted from the larva, while the imago may retain the pupal infection or may possibly acquire it *ab initio* by ingesting contaminated food.

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Further references will be found in some of the memoirs cited.

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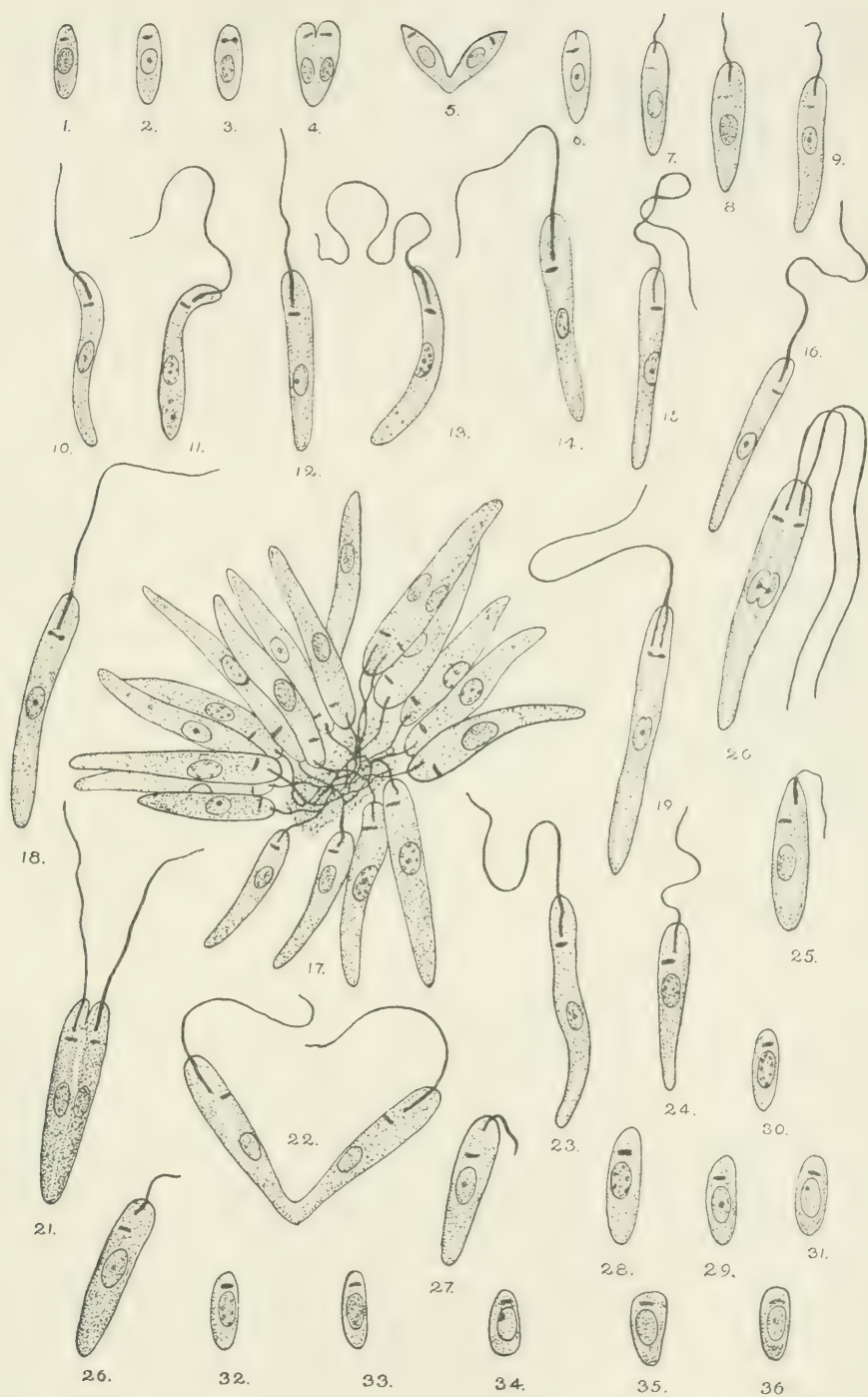
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EXPLANATION OF PLATE XLI

All the figures were outlined with an Abbé-Zeiss camera lucida, using one-twelfth inch oil immersion lens and compensating ocular No. 8. Magnification 1,500 diameters, approximately.

- Figs. 1-22. Illustrate the preflagellate and flagellate stages found in the larva.
- Figs. 1-9. Illustrate the development of *Herpetomonas stratiomyiae* from the preflagellate to the flagellate stage.
- Fig. 1. Preflagellate form with nuclear chromatin evenly distributed.
- Fig. 2. Preflagellate with nucleus showing a karyosome.
- Figs. 3-5. Stages in the division of a preflagellate.
- Fig. 3. Preflagellate with blepharoplast dividing.
- Fig. 4. Form with blepharoplast and nucleus dividing.
- Fig. 5. Separation of the daughter organisms.
- Figs. 6-9. Show the development of a flagellum and assumption of the flagellate form.
- Figs. 10-16. A series of flagellates showing variation in size and appearance.
- Figs. 11, 13. Show flagellates containing chromatoid granules.
- Fig. 17. An aggregation rosette of flagellates of different ages, attached by their flagella to a piece of débris.
- Figs. 18-22. Stages in the division of flagellates.
- Fig. 18. Parasite showing constriction of blepharoplast.
- Fig. 19. Parasite with flagellum splitting.
- Fig. 20. Form with flagella and blepharoplast separate and nucleus dividing.
- Fig. 21. Commencement of division of the general body cytoplasm.
- Fig. 22. Daughter organisms almost separate.
- Figs. 23-36. Illustrate post-flagellate stages obtained from larvae and pupae.
- Figs. 23-25. Transitional forms between flagellate and post-flagellate stages, as obtained from the haemocoel of the larva.
- Fig. 26. Similar transitional flagellate from the hind gut of a larva.
- Figs. 27-31. Post-flagellates from pupae.
- Figs. 32-36. Post-flagellates from the hind gut of a larva.



H. B. F. et A. P. del.

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